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Distribution of the *Medea* factor M^4 in populations of *Tribolium castaneum* (Herbst) in the United States

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Abstract

The distribution of the maternally acting, selfish gene *Medea*^{4} (M^4) was determined in populations of the red flour beetle, *Tribolium castaneum* (Herbst), collected in the southern and midwestern United States. We found clear evidence for the existence of two major regional subpopulations, with a boundary that roughly corresponds to 33°N latitude. All 26 strains collected in 10 states north of this latitude were homozygous for the M^4 allele, while only two of 29 strains collected in six states south of this latitude were homozygous for the allele. Of the remaining 27 southern strains, 21 lacked the M^4 allele entirely, while six contained a mixture of M^4 and non-M^4 alleles. This is the first evidence of either the existence of biotypes or the presence of major barriers to gene flow in wild populations of this ubiquitous insect species. Published by Elsevier Science Ltd.

Keywords: *Tribolium castaneum*; Selfish gene; *Medea*; Biotype; Population genetics

1. Introduction

Medea factors are maternally-acting, selfish genes that operate by maternal kill of hatchlings, in combination with zygotic rescue from the maternal lethal effect (Beeman et al., 1992). Both activities are dominant and are tightly linked on the same chromosome. Each *Medea* factor encodes both activities, apparently in a single genetic locus. "Maternal kill" refers to the fact that a lethal *Medea* gene in the mother causes the death of some of her progeny, but has no effect on the mother herself. "Zygotic rescue" refers to the fact that copies of the maternally lethal *Medea* gene inherited by the progeny can protect those progeny from maternal kill. It is this self-rescuing property of *Medea* factors that accounts for their "selfish" behavior, and explains why they are

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predicted to be invasive in populations (Wade and Beeman, 1994). To our knowledge, maternally acting selfish genes have not been reported in any invertebrate species outside the genus *Tribolium*.

Previously it was reported that the two unlinked, autosomal *Medea* factors, M^1 and M^4, were widespread in world populations of the red flour beetle, *Tribolium castaneum* (Herbst) (Beeman and Friesen, 1999). We also noted a non-random distribution of both factors. M^1 was found to be prevalent in Asian, African and South American populations, but absent in populations from Australia, India, Europe or North America, while M^4 was prevalent in all regions tested except for India and Australia. In that report a distinct regional non-uniformity in the distribution of M^4 in North American populations was also noted. M^4 factors were found in all 11 beetle strains originating from the midwest or northern plains, but in none of the five strains originating from the Gulf coastal region. These results suggested that *Medea* factors could be useful indicators of population structure in the red flour beetle. In the present work, the regional distribution of M^4 in the United States is examined in greater detail by expanding the analysis to include a total of 55 strains originating from N latitude 27–45° (the Gulf coast to Minnesota) and from W longitude 80–102° (South Carolina to Texas).

2. Materials and methods

2.1. Description of strains

Field strains used in this study were collected from farms, country elevators, mills, grain or peanut companies or (for some of the southern locations) terminal elevators. Most strains were collected between 1993 and 1995, and all strains were obtained directly from the primary collector. Starter cultures consisted of 5–50 adult beetles from each original collection, with the exception of strain #257 (see Table 1). Prior to screening for M^4, all strains were confirmed to be non-M^1 using previously described methods based on genetic mapping (Beeman and Friesen, 1999) (data not shown). The standard laboratory strains M^4 and GA-1 have also been described (Beeman and Friesen, 1999). The former strain is identical to the "M^4 *au*" strain in the work cited. The latter strain is devoid of M^4 or other *Medea* factors. Unless otherwise indicated, the term "*Medea*" as used in this work refers only to the M^4 locus. Beetles were reared at 30°C on whole-wheat flour fortified with 5% v/v brewers' yeast.

2.2. Diagnosis of M^4 genotype

M^4 genotypes were determined by testing for the presence of M^4-associated zygotic rescue activity in field strain males. $M^4/+$ females show normal, 1:1 segregation of the M^4 and + alleles during oogenesis. Thus, if such females are crossed with $+/+$ males, approximately 50% of the progeny will be genotypically $+/+$ and will be killed as hatchlings by the maternal lethal effect of M^4. If the $M^4/+$ females are instead crossed with homozygous M^4 males, all progeny will be rescued from the maternal lethal effect by the zygotic protective action of the paternally derived M^4 allele (half are also protected redundantly by a maternally derived M^4 allele). Standard, virgin $M^4/+$ females were generated from a mass cross of virgin M^4 females with GA-1 males. For most tests, at least five single-pair crosses were set up between field strain males 1–6 weeks of age and

Table 1
M^4 diagnosis in midwestern and Gulf coastal populations of *Tribolium castaneum*

State of origin	Strain[a]	μ[b] ± s.d.	N	*Medea* type	Mann/Whitney[c] statistic	
					+/+	M^4/M^4
GA	+/+	52±9	5	+	$P\equiv1.0$	0.003
—	M^4/M^4	94±5	9	M	0.003	$P\equiv1.0$
MN	32	97±2	6	M	0.006	0.135
	63	87±15	6	M	0.012	0.385
	225	93±4	5	M	0.009	0.687
WI	224	95±6	5	M	0.009	0.544
IA	31	97±2	5	M	0.009	0.172
IL	30	96±5	6	M	0.006	0.372
IN	236	98±2	5	M	0.008	0.133
	237	98±2	5	M	0.009	0.049
KY	226	92±13	5	M	0.009	0.459
KS	227	94±11	5	M	0.008	0.344
	235	100±0	2	M	0.051	0.056
OK	231&233	98±3	5	M	0.008	0.104
	234	99±1	5	M	0.007	0.017
	238	94±12	5	M	0.008	0.175
	242	100	1	M	—	—
	243&6	99±2	5	M	0.007	0.017
	247	98±1	5	M	0.008	0.076
AR	249	97±2	5	M	0.008	0.138
	253	100±1	4	M	0.013	0.022
	264	93±10	4	M	0.014	0.586
TX	37&38	48±4	10	+	0.357	0.000
	239	99±1	5	M	0.008	0.020
	240	98±2	5	M	0.009	0.049
	241	98±2	5	M	0.009	0.105
	244	98±2	5	M	0.009	0.105
	245	92±14	5	M	0.008	0.344
	250	*78*±12	5	Mixed	0.009	0.038
	252	*79*±26	5	Mixed	0.116	0.736
	255	56±7	10	+	0.806	0.000
	256	48±10	5	+	0.499	0.000
	259	98±2	5	M	0.009	0.069
	260&267	93±10	8	M	0.003	0.353
	262	38±10	4	+	0.085	0.005
	265	45±4	4	+	0.221	0.005
TX	266	55±4	2	+	0.881	0.012
	268	49±8	3	+	0.764	0.012
LA	254	*58*±14	10	Mixed	0.462	0.000
	261[d]	*80*±22	5	Mixed	0.075	0.460
	270[d]	37±6	5	+	0.347	0.003
	263	46±4	5	+	0.251	0.003
	257	*73*[e]±5	10	Mixed	0.002	0.000
MS	258	93±11	7	M	0.004	0.453
AL	28	47±11	6	+	0.221	0.005
	219	47±4	5	+	0.295	0.003

Table 1 (*continued*)

State of origin	Strain[a]	μ[b] ± s.d.	*N*	*Medea* type	Mann/Whitney[c] statistic	
					+/+	M^4/M^4
GA	207	45 ± 6	5	+	0.209	0.003
	211	44 ± 6	5	+	0.141	0.003
	212	48 ± 8	5	+	0.463	0.003
	215	45 ± 5	5	+	0.248	0.003
	216	46 ± 9	3	+	0.368	0.012
	218	*76* ± 14	5	Mixed	0.009	0.061
	220	44 ± 15	5	+	0.251	0.003
	221	49 ± 5	5	+	0.402	0.003
	223	48 ± 6	5	+	0.465	0.003
SC	209	45 ± 9	5	+	0.209	0.003
	213	49 ± 4	5	+	0.346	0.003

[a] +/+ = GA-1, a standard non-*Medea* laboratory strain. M^4/M^4 = mas p au, a standard *Medea* strain. Other strain numbers are laboratory designations.

[b] mean percentage survival of progeny of approximately five single-pair test-crosses of strain male × standard M^4/+ female. Based on approximately 50 eggs per single-pair cross.

[c] Probability that the mean differs from that of +/+ or M^4/M^4 by chance alone.

[d] Strain #261 and 270 are both from Crowley (different locations within the city), but of the two, only strain 261 appears to carry both M and non-M alleles.

[e] All 10 males tested were the F_1 progeny of a single original female. All 10 males appear to be heterozygous thus, the original female was probably homozygous for one allele and her mate homozygous for the other.

virgin M^4/+ females 2–6 weeks of age. After a premating interval of 3–7 days, the pairs were transferred to 0.7–1.0 cm^3 of flour/yeast medium that had been sifted through a 50-mesh US Standard sieve (297 μm apertures). They were allowed to mate and oviposit for 3 days at 30°C. Adults were then discarded, and eggs collected on a 50-mesh sieve, counted, and transferred to fresh flour/yeast medium. M^4-rescued progeny were allowed to develop for 3–4 weeks at 30°C, at which time most were last-instar larvae or pupae. They were then collected on a 25-mesh sieve (624 μm aperture) and counted. Homozygous M^4 and non-*Medea* (GA-1) males were similarly tested as controls. Most strains were tested within 6 months (1–4 filial generations) of field collection.

2.3. Statistical analysis

Means from percentage survival measurements were compared using the Mann–Whitney test (SYSTAT 8.0 for Windows, SSCP Inc., Chicago, IL).

3. Results and discussion

The results (Table 1, Fig. 1) confirm and extend our earlier observation that eastern United States populations of *T. castaneum* are divided into a northern and a southern subgroup, with a boundary at roughly 33° N latitude. Of the 26 strains collected north of this latitude, all were fixed

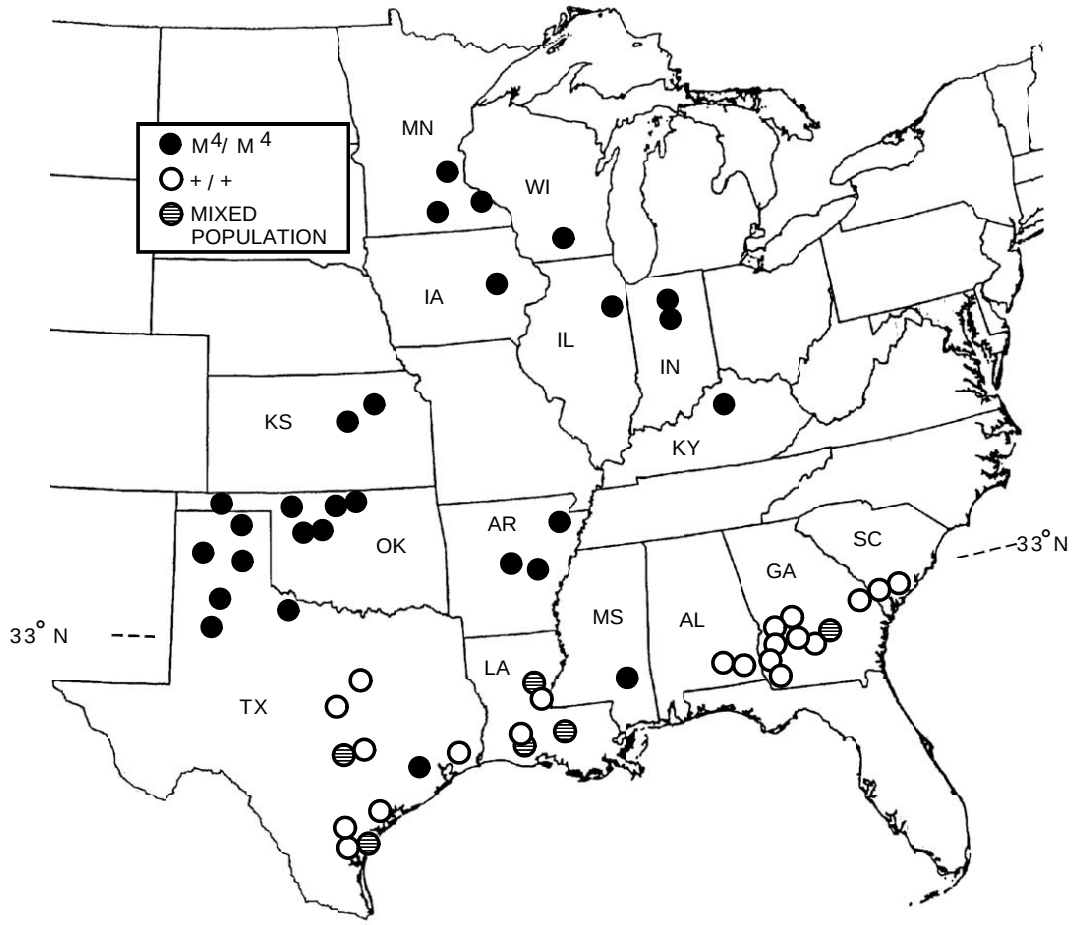

Fig. 1. Distributions of the M^4 Medea factor in *Tribolium castaneum* populations in the United States. Solid circles = homozygous M^4; open circles = homozygous non-*Medea*; hatched circles = mixture of M^4 and non-*Medea* alleles.

for the M^4 allele, i.e., all beetles tested from these strains appeared to be homozygous for M^4 because nearly 100% of the eggs hatched and survived to the last-instar larval or pupal stage. In sharp contrast, only two of the 29 strains collected south of 33° N (one each, from Texas and Mississippi) appeared to be fixed for M^4. In addition, six of the remaining 27 southern strains appeared to carry a mixture of M^4 and non-*Medea* alleles. These included one strain from Georgia, two from Texas and three from Louisiana. The remaining 21 southern strains were fixed for the non-*Medea* allele at the M^4 locus. All eight southern strains testing positive for M^4 were collected in facilities that received shipments of grain from sources north of the 33rd parallel. *Medea* diagnoses were confirmed by detection of *Medea*-killed first instar larvae. No dead hatchlings were found in any test vials representing the region north of the 33rd parallel, whereas such larvae were found in all vials examined from tests of southern strains (exceptions: strains 258 and 260, see Table 1).

This striking dichotomy between northern and southern populations of *T. castaneum* is the first compelling evidence for the existence of genetic races of this species. In general, stored-product insects are regarded as cosmopolitan, and barriers to gene flow within contiguous infested regions are considered to be minimal. We know from theoretical considerations (Wade and Beeman, 1994) and from laboratory observation (Beeman et al., 1992 and unpublished) that *Medea* alleles tend to spread into naïve populations and to suppress non-*Medea* alleles. The fact that not a single, non-*Medea* allele was found in any of the northern strains testifies to the effectiveness of this suppression or to the absence of south-to-north migration of *T. castaneum* in North America. Apparently some north-to-south migration does occur, as evidenced by the presence of *Medea* alleles in a few southern intermediary or port facilities that receive shipments of unprocessed corn or other grains from the north. However, the fact that *Medea* alleles are largely absent south of the 33rd parallel in spite of periodic introduction via commerce suggests that these two regional biotypes are predominantly non-intermingling and that significant barriers to population mixing must exist.

Tribolium castaneum is known to be a pest of corn, wheat, rice and peanuts, and is commonly found in farm storages of these and other commodities, as well as in elevators, mills and warehouses that house these products or their derivatives. The northern areas from which *Medea* biotypes were collected generally correspond to the corn belt and the winter wheat regions of the midwest. The non-*Medea* biotype was generally collected from the rice and peanut-growing regions of the gulf coastal states. The fact that raw peanuts and unprocessed rice are rarely shipped north from the gulf coastal region could contribute to the apparent lack of south-to-north migration of this insect. However, correlation between *T. castaneum* biotype boundaries and crop production boundaries are somewhat indistinct, and there is no obvious and compelling reason why such crop production boundaries should be associated with restriction of population fluxes, or should prevent intermingling of northern and southern biotypes.

The existence of two distinct races over such a large geographical range could be explained by either of two alternative scenarios. One possibility is that the current range of *T. castaneum* in the eastern United States was established after two separate introductions, one involving M^4 which spread across the midwest, and another involving non-M^4 which spread across the Gulf coast. These introductions must have been recent enough, the subsequent range expansions gradual enough, and barriers to intermingling strong enough, that the genetic evidence for these events is still apparent. The self-selecting property of *Medea* would ensure its maintenance at a high frequency in the midwest, whereas a low mutation rate, the gradual nature of *Medea* invasiveness, the recency of colonization, and perhaps even the existence of barriers to migration would ensure a continued high frequency of non-*Medea* in the southern coastal region. This situation could be confirmed by DNA sequence comparisons among midwestern and southern strains using sequences from or near the M^4 locus. The *Medea* gene M^1 has recently been isolated by positional cloning (unpublished data), and a similar approach is planned for isolating M^4. Under the "two introductions" scenario many other genetic loci might also be expected to show regional dimorphism.

A second possibility is that the original introductions were all M^4, but subsequently the M^4 allele was lost in the southern regions, perhaps because it is better suited to a temperate climate. This seems less likely than the "two introductions" scenario, in view of the widespread occurrence of M^4 in the tropical regions of Africa, South America and Southeast Asia. Once

again, DNA sequence comparisons, both genome-wide and in the vicinity of the M^4 locus might be pertinent.

There has been some recent interest in the possibility of genetic fingerprinting of stored-product insect pest populations, including *T. castaneum,* and also of other species for which less sequence information is available (Braet et al., 1995; Stuart et al., 1996; Dowdy and McGaughey, 1996; Brown et al., 1997; Silvain and Delobel, 1998). The present report on biotype analysis in *T. castaneum* hints at the untapped potential for using genetic and DNA markers to better elucidate regional population structure and perhaps even provide insights into more localized population fluxes and infestation sources for these insects. Our whole genome map of *T. castaneum* (Beeman and Brown, 1999, and unpublished data) represents a large source of polymorphic loci upon which to base future DNA fingerprint analysis of *Tribolium* populations.

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